# THE PERMEABILITY OF THE SENSORY PEGS ON THE ANTENNAE OF THE GRASSHOPPER (ORTHOPTERA, ACRIDIDAE)

ELEANOR H. SLIFER

*Department of Zoology, State University of Iowa, Iowa City, Iowa, and the Marine Biological Laboratory, Woods Hole, Mass.*

In 1906 Röhler described three types of sense organs—pegs (*Kegel,* sensilla basiconica), pit pegs (*Grubenkegel,* sensilla coeloconica) and bristles (*Sinnesborsten,* sensilla chaetica)—which are present on the antennae of a grasshopper, *Acrida turrita* (Linnaeus).[1] Jannone (1940) states that the antenna of another species, *Dociostaurus maroccanus* (Thunberg), is provided with the same kinds of sense organs. He counted 125 pit pegs on the antenna of a first instar female and found between 470 and 490 on the antenna of an adult female. Eiben (1949) showed that similar structures occur on the antennae of *Melanoplus differentialis* (Thomas). He recorded the number of each type present on the antennae of each of the six nymphal instars and of the adults of this species and found that there is a five-fold increase in the total number of these sense organs during post-embryonic development. In addition to the sensory structures found by Röhler others have been described on the surface and inside the antennae of grasshoppers (McIndoo, 1920; Eggers, 1924; Slifer, 1936; Jannone, 1940; McFarlane, 1953) but these need not be considered here.

Of the three types of sense organs which were found by Röhler on the antenna of the grasshopper the basiconic pegs are most numerous. Sensilla of this kind are known to be present in many species of insects and are generally considered to be chemoreceptors (Snodgrass, 1926, 1935; Frings and Frings, 1949; Roth and Willis, 1951; Hodgson, 1953 and others) but, as Dethier (1953) says of these and related structures (p. 546): "Nothing is known concerning the chemical or physical properties of the cuticle surmounting these receptors." Richards' (1952) studies on the antennae of the honeybee have recently supplied some information on the properties of the cuticle of fixed and sectioned sense organs. It is the purpose of the present paper to show that in the living grasshopper the tip of certain of the sensory pegs is permeable to aqueous solutions of a dye.

## Materials and Methods

The species of grasshoppers which were examined in the living condition represent the three major North American subfamilies (Acridinae, Oedipodinae and Cyrtacanthacridinae) and included male and female *Orphulella pelidna* (Burmeister), *Dissosteira carolina* (Linnaeus), *Psinidia fenestralis fenestralis* (Serville), *Melanoplus differentialis differentialis* (Thomas), *Melanoplus femur-rubrum* (DeGeer) and *Melanoplus mexicanus mexicanus* (Saussure). Some of these were raised in the laboratory and others were caught in the field. Newly-hatched nymphs of *Melanoplus mexicanus mexicanus* were used in certain experiments and newly-molted adults of several species in others. Preserved specimens of adult *Acrida bicolor* (Thunberg),[2] *Dissosteira carolina, Locusta migratoria migratorioides*

[1] Known to Röhler as *Tryxalis nasuta* L.

[2] The preserved specimens of *Acrida bicolor* and *Locusta migratoria migratorioides* were kindly sent to the writer by Dr. B. P. Uvarov of the Anti-Locust Research Centre in London.

(Reiche and Fairmaire), *Melanoplus femur-rubrum femur-rubrum* and *Melanoplus mexicanus mexicanus* were also studied.

Of several dyes tried a 0.5% aqueous solution of acid fuchsin was found to be especially useful. It is a vivid stain and can be detected when present in minute quantities. When used as described below it has no toxic effects and nymphs immersed in it for an hour recovered completely after removal from it. The method used to demonstrate the penetration of the dye was simple. If the individual to be tested was small its head was removed, wrapped in a bit of absorbent cotton and the whole placed in the dye. For larger insects the antennae were severed at the base, wrapped in cotton and immersed in the stain. The cotton prevents the specimen from rising to the surface of the solution where it would, otherwise, float. Care must be taken that no air bubbles are trapped in the cotton for they may prevent the stain from reaching all parts of the antennae. After a suitable interval—a few minutes to several hours—the head or antenna was removed and dipped very rapidly, and in turn, into distilled water, 70% alcohol and absolute alcohol to wash off stain which was clinging to the surface. The specimen was then placed in n-butyl alcohol or dioxan where it was left for five minutes or longer depending upon its size. Here the antennae were removed from the head if this had not been done earlier. Toluol was used as a final clearing agent and the antennae were mounted in a synthetic resin (Harleco H. S. R.) which was dissolved in toluol. In using this method it is of the first importance that passage from the stain to n-butyl alcohol or dioxan be very rapid for the fuchsin is lost quickly if there is any delay. Dehydrating agents in which acid fuchsin is soluble must be avoided and the same applies to clearing and mounting media. Before any other reagent is substituted for one of those used here a sample should first be tested by adding a small amount of the dry, powdered dye to it. Finally, it should be noted that special difficulties will be encountered when this method is used for studying large structures which have much soft tissue or body fluid associated with them. The water in this tissue or fluid may dilute and carry the stain away with it while the specimen is being dehydrated.

## Results and Discussion

The basiconic or peg-like sensilla on the antennae of the grasshoppers studied may be subdivided into at least three kinds:[3] (1) long, slender pegs with a narrowly-rounded tip (Figs. 1 to 7, a), (2) short, stout pegs with a broadly-rounded tip (Figs. 1 to 7, b), and (3) short, slender pegs with a pointed tip (Figs. 1 to 7, c). Of these only the first are permeable to acid fuchsin. The other two are unaffected by the stain. No clue as to their function has been obtained, and they will not be considered further here.

[3] Snodgrass (1935, p. 519) discusses variations in basiconic sensory structures as follows: "Sensory pegs and cones are innervated hairs reduced in size, and there is no sharply dividing line between sensilla trichodea and sensilla basiconica, either in the character of the external parts or in the structure of the internal parts. In a typical sensillum basiconicum the external process is a small peglike or conical structure (Fig. 269 A, *Pg*). The walls of the process are thick or strongly sclerotic in some cases, while in others they are thin and transparent, or the process may terminate in a delicate membranous cap."

The extent to which the stain penetrates the long, slender pegs which were described in the preceding paragraph, depends largely, although not entirely, upon the time of exposure. If the antenna is left for a short time in the dye only the extreme tip of each peg is colored and examination with an oil immersion lens may be necessary to detect the minute red spots. This indicates that the whole outer

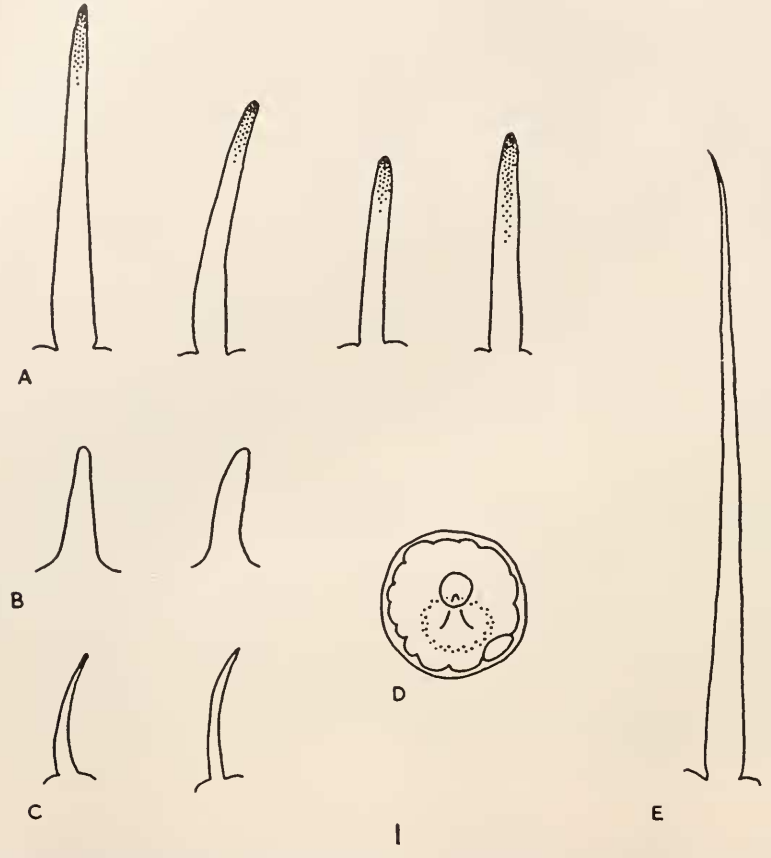
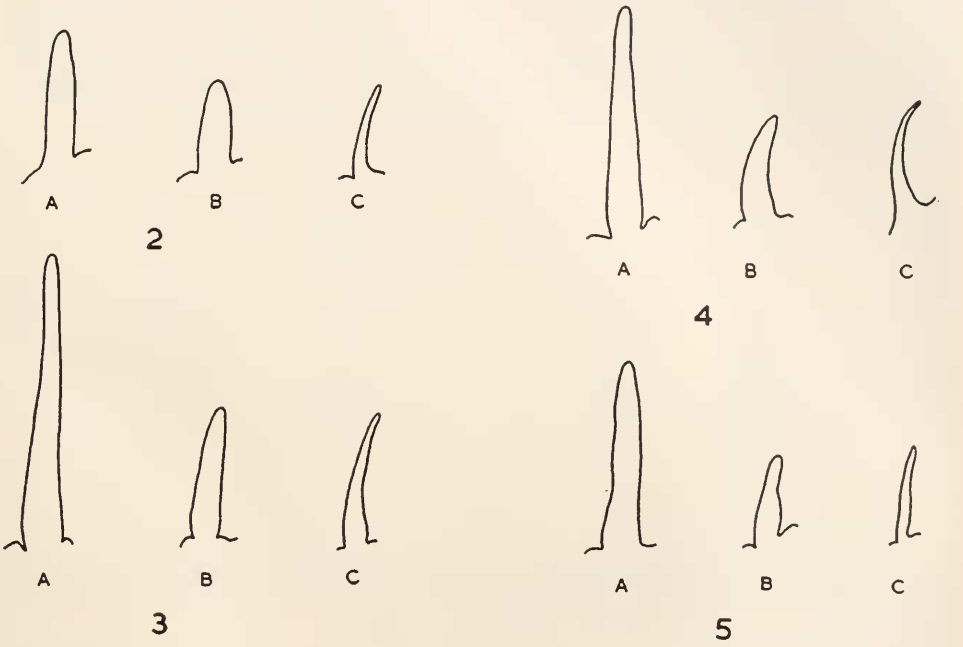



FIGURE 1. Sensory structures from surface of the antenna of an adult female *Melanoplus differentialis differentialis* which, nineteen hours after the final molt, was treated for 30 minutes with an aqueous solution of acid fuchsin. A, long, slender basiconic pegs which are permeable to the dye at their tips; stippled area shows extent of penetration of the stain during 30 minutes; B, short, stout basiconic pegs which are unaffected by the dye; C, short, slender basiconic pegs which are unaffected by the dye; D, surface view of coeloconic peg; small, brownish, oval mass of unknown origin and identity which is commonly present in such pits shown at lower right; E, sensory bristle which is unaffected by stain. × 1100.

surface of the peg is waterproof except at the tip. Here the usual waxy or lipoid layers of the cuticle must be missing. The inner, permeable layers of the cuticle extend across the tip and there is no actual opening or pore. In antennae which have been left longer in the stain the dye will be found to have traversed the permeable cuticle at the tip and to have entered the central cavity or core of the peg. The extent of this cavity can be seen in antennae which have been allowed to dry before

being mounted in resin, for the air-filled core of the peg then appears black under the microscope. Since the cavity of the peg in the living insect contains either fluid or cytoplasm, which extends up into it from the cellular layer below, the passage of the dye is more rapid down the central core. At the same time, but a little more slowly, diffusion occurs laterally from the core through the inner, cuticular layers of the peg. After very long exposures the entire peg is colored and the dye may reach the interior of the antenna itself.



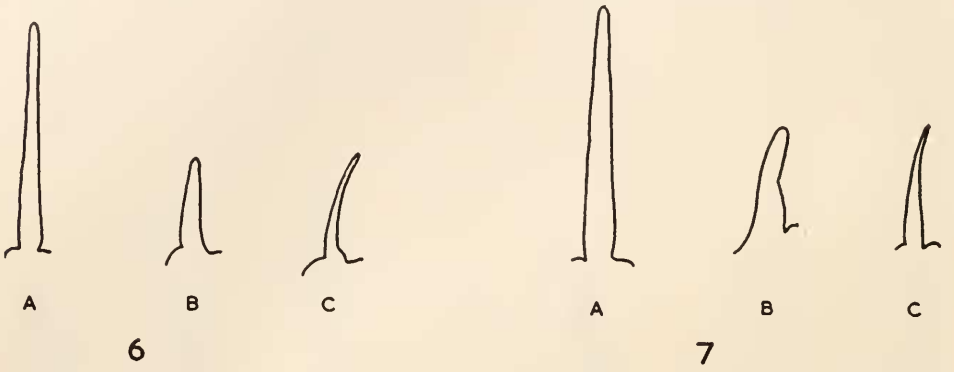

FIGURES 2 TO 5. Basiconic pegs from the surface of the flagellum of the antennae of adults of four species of grasshoppers. Figure 2, male *Acrida bicolor;* Figure 3, male *Orphulella pelidna;* Figure 4, female *Dissosteira carolina;* Figure 5, male *Locusta migratoria migratorioides.* A, long, slender peg which is permeable at the tip; B, short, stout peg which is unaffected by dye; C, short, slender peg which is unaffected by dye. × 1100.

The tips of the long pegs stain with great regularity and the rest of the surface of the antenna shows no trace of the dye except in those regions where an obvious injury has occurred. To eliminate all possibility that the tips of the pegs stained because they had been worn or abraded tests were made with the antennae of adults which had just molted. The results with these were the same as had been obtained with older animals. On the antennae of a freshly-molted individual, where the cuticular surface is still perfect, only the tips of the long, basiconic pegs are colored by the dye.

These pegs are permeable to acid fuchsin in the newly-hatched grasshopper. To establish this point nymphs of *Melanoplus mexicanus mexicanus* which had left the egg a few moments before and had just shed the cuticle with which they hatch were treated with fuchsin. The tips of their long basiconic pegs stained brilliantly but

the entrance of the dye into the cavity of the peg was slower than it is in the adult. It was interesting to find that these sensilla in the newly-hatched nymph are of approximately the same size as are those of the adult (Figs. 6 and 7) although, as Jannone (1940) and Eiben (1949) have shown, antennal sense organs are much fewer in number in the former. Why penetration of the dye should be slower in the young insect is not known but the size of the colored area at the tip suggests that the permeable surface is even smaller than it is in the older animals.

The bristles or sensilla chaetica described by Röhler for *Acrida turrita* are also present on the antennae of the species studied here (Fig. 1,e) but they are few in number and are located only on the proximal segments. Such bristles are usually believed to have a tactile function. They are entirely unaffected by the dye in newly-molted individuals, but these long bristles are often found to be damaged in older animals and the stain then enters rapidly through the broken end.




FIGURES 6 AND 7. Basiconic pegs from surface of flagellum of antenna of newly-hatched and of adult *Melanoplus mexicanus mexicanus*. Figure 6, newly-hatched; Figure 7, adult male. A, long, slender peg which is permeable to dye; B, short, stout peg which is unaffected by dye; C, short, slender peg which is unaffected by dye. × 1100.

As Röhler reported in 1906 another type of sense organ present on the grasshopper antenna is the pit peg or sensillum coeloconicum. There is considerable evidence that coeloconic sensilla in other insects are chemoreceptors. In these sense organs, in the grasshopper, the peg is located at the bottom of a small, globular pit which opens to the surface through a still smaller hole (Fig. 1, d). The cavity, in life, is filled with air and because of this it has not been possible to demonstrate, with the method outlined above, that the tip of the peg is permeable. When the antenna is immersed in water or in an aqueous solution of acid fuchsin the pits remain filled with air and this prevents the fluid from coming into contact with the tip of the peg. The air bubbles in the pits are easily seen under the microscope. No method for removing these bubbles, which may not be suspected of damaging or altering the tip, has yet been devised. Attempts to remove the bubbles with a vacuum pump and with a detergent solution were made but the results were not satisfactory. It is possible that dye might be placed in the pit with the aid of a microdissection syringe but this was not tried. Antennae from animals which have been fixed in Bouin's solution and preserved in 70% alcohol provided some information concerning the permeability of the pegs. In these the pits have filled with

alcohol and when such antennae are placed in fuchsin, as were the living antennae, the dye replaces the alcohol in the pits and the tips of the pegs then take up the stain just as do the tips of the long, basiconic pegs of the same specimen. It will be noticed in such preparations that the stain penetrates the latter more rapidly than it does in fresh material but the manner of entry is the same. From these observations we may conclude that the tips of the coeloconic pegs also differ from the general cuticular surface in respect to permeability and that it is probable, although, of course, not yet proved, that they, too, would be permeable to an aqueous solution of fuchsin in the living condition were it possible to bring the dye into contact with them.

Since the relatively large molecule of acid fuchsin penetrates the tip of the living basiconic peg so readily there can be no doubt that this region is also permeable to water and it is highly probable that a great variety of other substances could also be shown to pass through it if methods suitable for their detection were applied. The results reported here, then, strongly support the conclusions of many previous investigators who, on other grounds, and with other species of insects, have believed certain basiconic pegs to be chemoreceptors, hygroreceptors or both. The results obtained with fixed material suggest that the coeloconic pegs may have a similar function or functions although the evidence is less reliable than it is for the long, basiconic pegs.

Preliminary examinations of other parts of the body of the grasshopper have shown that long, slender pegs of the type present on the antenna occur also in other regions, although more sparsely, and that they, too, have a permeable tip.

Whether insects other than grasshoppers also possess basiconic pegs which are permeable to water and dyes is not known with certainty at present. A few adults belonging to other orders (Collembola, Thysanura, Dermaptera, Isoptera, Neuroptera, Coleoptera, Hymenoptera and Diptera) were tested with interesting but inconclusive results. In some specimens definite and regular staining occurred but since only a few tests were made and since the individuals used were of unknown age and past history it is possible that the tips of the sensilla which stained may have previously been damaged. For critical work the animals should be freshly-molted or, at least, known never to have been in contact with a surface or object which might injure the tips of the pegs. These cursory tests, however, brought out several of the difficulties which may be encountered when insects other than grasshoppers are studied. In some the covering of long, close-set hairs retains a film of air which prevents the stain from reaching the pegs even though the specimen is wrapped in cotton which is covered by the dye solution. In others the cuticle is heavily pigmented and it is impossible to decide whether or not any staining has occurred. In still other individuals faint staining was apparent only after many hours exposure to the dye which suggests that the rate of penetration must be extremely slow in these forms. In certain species large, thin-walled pegs were found barely tinged with pink. Here, seemingly, small amounts of the dye had penetrated and then diffused through the fluid contents of the peg. Finally, it should be emphasized that failure to stain with acid fuchsin does not mean that the structure tested is impermeable to all materials. It may still be permeable to water and to substances other than the dye used here. Clear-cut, positive results, such as are given by the long, slender pegs of the grasshopper antenna, lend very strong support to the idea that these structures, in this insect, serve as chemoreceptors, as

hygroreceptors, or, perhaps, as both. Negative results, on the other hand, prove only that, under the conditions of a particular experiment, the structure tested is either impermeable to acid fuchsin in detectable amounts or that any dye which did penetrate was later lost.

## Summary

1. When an aqueous solution of acid fuchsin is applied to the surface of the living antenna of a grasshopper the dye enters the tips of the largest of three types of basiconic sense organs while the other two types are unaffected.

2. The permeability to water and to dye of these long basiconic pegs on the antenna of the living grasshopper strongly supports the conclusions of earlier workers with other insects that such structures may serve as chemoreceptors, as hygroreceptors, or as both.

3. The permeability to water and to dye of the pegs of the coeloconic sense organs on the surface of the antennae of preserved grasshoppers suggests that these, too, may function as chemoreceptors, as hygroreceptors, or as both but the evidence is less satisfactory than it is for the long basiconic pegs.

4. *Positive results* with the staining method described in the present paper indicate that the structure tested is permeable to acid fuchsin and to water and, probably, to many other substances as well. *Negative results* mean either (1) that the structure is completely or nearly impermeable to acid fuchsin or (2) that any of the stain which did enter was lost in later handling.

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